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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PTO-PAT-Email@rfem.com

Office Action Summary	Application No.	Applicant(s)	
	10/666,535	ICHIKAWA ET AL.	
	Examiner	Art Unit	
	David S. Romeo	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX*(6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *0507*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 04/20/2007 has been entered.

Claims 1–14 are pending and being examined.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1 and 3 are rejected under 35 U.S.C. 102(b) as being anticipated by Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527).

U. S. Patent No. 7,235,527 is a national stage entry of PCT/JP96/01062. PCT/JP96/01062 was published in Japanese as WO 96/33215 on 10/24/1996. The examiner relies on U. S. Patent No. 7,235,527 as an English translation of WO 96/33215.

Makishima ('527) discloses that injectable preparations of human MP52 can be formulated, for example, in the form of injectable powders. In that case, the powders can be prepared by adding one or more of suitable water-soluble excipients such as mannitol, sucrose, lactose, maltose, glucose, fructose and the like, to human MP52, dissolving the mixture in water, dividing it into vials or ampoules followed by lyophilizing and hermetically sealing. (Column 3,

last full paragraph). The human MP52 is produced by means of a genetic engineering technology (column 2, full paragraph 3). Accordingly, Makishima (WO 96/33215) teaches a lyophilized composition of MP52, which comprises MP52 and mannitol, wherein said MP52 is produced by means of a genetic engineering technology. Makishima's (WO 96/33215) disclosure is tantamount to the disclosure of a process for the preparation of a lyophilized composition comprising MP52 and mannitol.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1–7 and 12–14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) as applied to claims 1 and 3 above, and further in view of Ron (U. S. Patent No. 5,171,579) and Avis (1990).

Makishima (WO 96/33215) teaches a process for the preparation of a lyophilized composition comprising MP52 and mannitol, wherein said MP52 is produced by means of a genetic engineering technology, and the lyophilized composition produced as a result of the process, as discussed above. Makishima (WO 96/33215) does not teach, only in the sense that Makishima (WO 96/33215) does not anticipate, mixing MP52 and mannitol at a weight ratio of 1:5-50.

Ron teaches that osteogenic proteins can be utilized in the form of a pharmaceutically acceptable solution (including reconstitution from a lyophilized form). It is optimal to solubilize

the osteogenic protein at concentrations of at least about 2 mg/ml, preferably about 4 mg/ml, so that a pharmaceutically effective amount of protein can be delivered without undue volumes of carrier being necessary (column 2, lines 22-29). Ron teaches that additional optional components useful in the practice of the subject application include, e.g. cryogenic protectors such as mannitol (to protect from degradation during lyophilization), preservatives, antioxidants, etc. (column 4, lines 45-48). Ron's preferred osteogenic protein is recombinant BMP-2, but any isolated or recombinant osteogenic proteins of the TGF-β family would be similarly useful (column 1, lines 13-34 and 63-65; and column 2, lines 12-14 and 22-24).

Avis teaches that the particular advantages of freeze-drying (lyophilization) are ease of processing a liquid, pharmaceuticals can be stored in a dry state in which there are relatively few stability problems, the products are often more soluble and/or more rapidly soluble, and dispersions are stabilized throughout their shelf-life (page 1565, paragraph bridging columns 1-2, through column 2, full paragraph 1). Avis teaches that mannitol has been found to be most useful to increase the solids content of the original solution to between approximately 5 and 25% so that the freeze-dried product plug occupies essentially the same volume as that of the original solution (page 1566, column 2, full paragraphs 1-3). A 5 to 25% mannitol solution contains 50 to 250 mg mannitol per ml.

Ron and Avis do not teach mixing MP52 and mannitol at a weight ratio of 1:5-50.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to prepare of a lyophilized composition comprising MP52 and mannitol, as taught by Makishima (WO 96/33215), and to modify that teaching by mixing the osteogenic protein at a concentration of at least about 2 to 4 mg/ml, as taught by Ron, with mannitol at a

concentration of 50 to 250 mg/ml, as taught by Avis, prior to lyophilization with a reasonable expectation of success. In so doing, one would add the mannitol to an aqueous solution of MP52 and obtain a solution comprising MP52 and mannitol, wherein the concentration of mannitol is with in the range 0.5-5% (w/v). Lyophilization of such a solution would yield a lyophilized composition comprising MP52 and mannitol, wherein said MP52 and mannitol are mixed at a weight ratio within the range of 1:5-50. One of ordinary skill in the art would be motivated to make this modification because it is optimal to solubilize osteogenic proteins at a concentration of at least about 2 to 4 mg/ml so that a pharmaceutically effective amount of protein can be delivered without undue volumes of carrier being necessary, and because cryogenic protectors, such as mannitol, protect from degradation during lyophilization. One of ordinary skill in the art would have been further motivated to make this modification because the particular advantages of freeze-drying (lyophilization) are ease of processing a liquid, pharmaceuticals can be stored in a dry state in which there are relatively few stability problems, the products are often more soluble and/or more rapidly soluble, dispersions are stabilized throughout their shelf-life, and because mannitol has been found to be most useful to increase the solids content of the original solution to between approximately 5 and 25% so that the freeze-dried product plug occupies essentially the same volume as that of the original solution. A solution comprising 2 to 4 mg/ml MP52 and 50 to 250 mg/ml mannitol is a solution comprising MP52 and mannitol in the range of 1 : 5-50 (ratio by weight). A lyophilized form of said solution is a composition comprising MP52 and mannitol in the range of 1 : 5-50 (ratio by weight).

Regarding the limitations "prevention of coloration," "prevention of shrinking," and

"prevention of aggregation," *prima facie* obviousness does not require that the prior references suggest combining their disclosure for the same reasons that Applicants combined them. Ron specifically suggest using mannitol as a cryoprotectant to prevent degradation of BMPs during the lyophilization process, which fairly suggest using mannitol during lyophilization of MP52 (a member of the same osteogenic protein family as BMPs). It would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to prevent degradation as much as possible for as long as possible in order to have an as potent as possible for as long as possible an MP52 biologic or pharmaceutical, which is motivation to prevent degradation by adding mannitol in accordance with the teachings of Ron. Furthermore, these results would have been unexpected to one skilled in the art. Discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art. According to Avis, one skilled in the art of lyophilization typically considers "the nature and stability characteristics required during the liquid state, both freshly prepared and when reconstituted before use, [as well as] the characteristics of the dried plug" (Avis, page 1566), when formulating a pharmaceutical or biological product, i.e., "whether the lyophilized substance will be dull or spongy or sparkling and crystalline, firm or friable, expanded or shrunken, etc." (*id.*). It is fair to say that Avis identifies the choice of excipient as a "result effective variable." Identification of mannitol as the ideal lyophilization excipient for MP52 would have been within the ordinary skill of the art.

The invention is prima facie obvious over the prior art.

Claims 7–14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Makishima

(WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and further in view of Ron (U. S. Patent No. 5,171,579) and Avis (1990) as applied to claims 7 and 12–14 above and further in view of Chang (J Pharm Sci. 1996 Dec;85(12):1325-30).

Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) further in view of Ron and Avis teach a solution comprising MP52 and mannitol and a lyophilized form of said solution, as discussed above. Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) in view of Ron and Avis do not teach a solution comprising MP52 and mannitol and a lyophilized form of said solution, wherein said solution comprises a surfactant.

Chang teaches that the addition of small amounts of surface-active agents protected proteins from both freeze- and surface-induced denaturation (Abstract). Freezing plays a crucial role in the damage incurred by proteins during freeze-drying (page 1325, left column, full paragraph 1). It has been found with a few proteins that low concentrations of surfactants (i.e., below the critical micellar concentration), which would not be expected to greatly alter the free energy of protein denaturation, provide a high degree of protection during freeze-thawing. Surfactants are known to stabilize proteins against surface-induced denaturation, so these results also suggest that the ice-water interface can contribute to freeze-induced protein denaturation. See page 1325, right column, full paragraph 1. Chang's results indicate that the freezing-induced denaturation is related to the exposure of proteins to an ice-water interface, so it seems rational to use surfactants as cryoprotectants (page 1327, right column, full paragraph 2). The capacity of 0.01% Tween 80 to protect proteins during freeze-thawing appears to be quite general because all of the model proteins were essentially completely protected (paragraph bridging pages 1327-1328). To determine how general this protective effect was, the influence

of several surfactants, with different chemical structures, on freeze denaturation of LDH was tested. All the tested surfactants protected LDH from precipitation during a quench-freezing process, even though the control frozen without a surfactant showed a significant increase in turbidity (Table 2). See page 1328, left column, full paragraph 1. The surfactants tested included Tween, Triton, and Brij. See page 1328, Table 2. This general stabilization of proteins during freeze-thawing by relatively low concentrations of surfactants strongly supports the contention that damage to proteins during freezing is due, at least to a large degree, to surface denaturation (page 1328, left column, full paragraph 2). In general, it appears that to obtain native, nonaggregated protein after freeze-drying and rehydration, it is necessary to develop a formulation that protects the protein during both freezing and drying. When 0.1% of Tween 80 was included in the protein solution, the soluble aggregate content decreased to 3%. Inclusion of 1% sucrose in the formulation, which is known inhibit unfolding of proteins during freeze-drying, led to slightly less protection, as reflected by a 8% aggregate content upon reconstitution. See paragraph bridging pages 1328-1329. To ascertain at what point during the freeze-drying and rehydration process Tween 80 was providing its beneficial effects, Chang conducted the following experiments. First, Chang found that the surfactant can prevent the formation of aggregates during rehydration. When the protein was freeze dried without surfactant but reconstituted with 0.1% Tween 80, the aggregate content was reduced to 23%. However, this protection was not sufficient to explain the reduction of aggregate content to 3% when Tween 80 was included prior to the freeze-drying. See page 1329, left column, full paragraph 1. Thus, for the rational design of stable protein formulations for freezing and freeze-drying, it is advantageous to include a surfactant, which apparently inhibits the denaturation of

proteins during freezing. A surfactant may only be sufficient to protect proteins during the freezing step. Another stabilizer, which is known to confer protection during drying (e.g., sucrose) will probably be needed to completely inhibit protein unfolding during freeze-drying. See page 1329, right column, last paragraph. Freeze-drying of IL-1ra solutions was performed as follows. An IL-1ra solution containing 2 mM potassium phosphate buffer, 3% mannitol, and 1 or 100 mg/mL of protein was prepared by dialysis. Mannitol was included as a crystalline bulking agent. Addition of 1% (w/v) sucrose or 0.1% (w/v) Tween 80 was made after the dialysis. Each vial was frozen by immersion into liquid nitrogen and loaded onto a shelf that was prechilled to -50 °C. After 1 h at -50 °C, the shelf temperature was increased to -15 °C at a rate of 1 °C/h. After an additional hour of incubation of the vials at this temperature, drying was initiated by decreasing the chamber pressure to 100 μm Hg. Then, the shelf temperature was increased to 25 °C at a rate of 1 °C/min. After completion of a 10-h drying cycle under vacuum the vial head spaces were filled with dry gaseous nitrogen. The vials were capped with stoppers until further analysis. See page 1326, left column, full paragraph 4. It is fair to say that Chang identifies the addition of surfactants and their combination with another stabilizer which is known to confer protection during drying, as a "result effective variable" for inhibiting protein denaturation during freeze-drying. Chang does not teach a lyophilized composition comprising MP52 and mannitol, a process for the preparation of said composition, a solution comprising MP52 and mannitol, or said solution further comprising a detergent.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to make teach a solution comprising MP52 and mannitol and a lyophilized form of said solution, as taught by Makishima (WO 96/33215) in view of

Makishima (U. S. Patent No. 7,235,527) and further in view of Ron and Avis, and to modify that teaching by adding a surfactant, as taught by Chang, with a reasonable expectation of success. One of ordinary skill in the art would be motivated to make this modification because for the rational design of stable protein formulations for freezing and freeze-drying, it is advantageous to include a surfactant, which apparently inhibits the denaturation of proteins during freezing.

The invention is prima facie obvious over the prior art.

Claims 7–10 and 12–14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and further in view of Ron (U. S. Patent No. 5,171,579), and Avis (1990) as applied to claims 7 and 12–14 above and further in view of Chang (J Pharm Sci. 1996 Dec;85(12):1325-30) and further in view of Hansen (U. S. Patent No. 6,586,574) and in light of the MeSH definition of "poloxamer."

Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and further in view of Ron and Avis and further in view of Chang teach a solution comprising MP52, mannitol, and a surfactant, as discussed above. Neidhardt in view of Ron and Avis and further in view of Chang do not teach a solution comprising MP52, mannitol, and a polyoxyethylene-polyoxypropylene copolymer.

Hansen relates generally to the stabilization of freeze-dried proteins. Hansen discloses that further stabilization of freeze-dried proteins can be obtained by the addition of surfactants, such as Tween and poloxamers (column 6, full paragraph 3). Poloxamers are polyoxyethylene-

polyoxypropylene copolymers, as evidenced by the MeSH definition of "poloxamer." The term "surfactants" generally include those agents, which protect the protein from air/solution interface-induced stresses and solution/surface induced-stresses (e.g. resulting in protein aggregation), and may include detergents such as polysorbate, poloxamer or polyethylene glycol, and the like. Optionally, concentrations from about 0.01% to about 1% (w/w) are suitable for maintaining protein stability, however, the levels used in actual practice are customarily limited by clinical practice. See column 5, full paragraph 2. Hansen does not teach a solution comprising MP52, mannitol, and a polyoxyethylene-polyoxypropylene copolymer.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to make a solution comprising MP52, mannitol, and a surfactant, as taught by Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and further in view of Ron and Avis and further in view of Chang, and to modify that teaching by making a solution comprising MP52, mannitol, and a poloxamer, as taught by Hansen, with a reasonable expectation of success. One of ordinary skill in the art would be motivated to make this modification because further stabilization of freeze-dried proteins can be obtained by the addition of surfactants, such as poloxamers.

The invention is prima facie obvious over the prior art.

Response to Arguments

Applicants argue that:

> The Avis reference provides a general discussion of lyophilization but has nothing to do with proteins in particular. Many substances are indicated as being possible agents to make the dried-product plug occupy essentially the same volume as that of the original solution. The disclosure of Avis would not have guided one skilled in the art to select mannitol for use with MP52 from the numerous recited substances. As discussed in prior responses, applicants have found that products used in the prior art were not successful

when used with MP52 and thus the use of specific substances is not predictable from the general disclosure in the cited prior art.

Applicants' arguments have been fully considered but they are not persuasive. The examiner considers applicants' arguments a piecemeal analysis of the Avis reference. Avis must be read, not in isolation, but for what it fairly teaches in combination with the prior art as a whole. According to Avis, one skilled in the art of lyophilization typically considers "the nature and stability characteristics required during the liquid state, both freshly prepared and when reconstituted for use, [as well as] the characteristics desired in the dried plug" (Avis, page 1566), when formulating a pharmaceutical or biological product. More to the point, Avis identifies the choice of excipient as a variable affecting the characteristics of the lyophilized product, i.e., "whether the lyophilized substance will be dull and spongy or sparkling and crystalline, firm or friable, expanded or shrunken, etc." (Avis, page 1566). It is fair to say that Avis identifies the choice of excipient as a result effective variable, and the identification of mannitol as the ideal lyophilization excipient for MP52 would have been within the ordinary skill in the art. Furthermore, patentability requires novelty and unobviousness in light of the prior art, not in light of what applicants knew and included in their patent application.

Applicants argue that:

> Ron does not cure the deficiencies in Avis as Ron does not suggest that mannitol is suitable for the use in a lyophilized product of MP52 either. Ron generally suggests that additional optional components such as cryogenic protectors might be useful. However, no examples were carried out in order to show that mannitol is in fact suitable in the connection with osteogenic proteins. Mannitol tends to form crystals during freezing and to leave the protein exposed (Williams NA, Lee Y, Polli GP, et al., "The Effects of Cooling Rate on Solid Phase Transitions and Associated Vial Breakage Occurring in Frozen Mannitol Solutions," J Parent Sci Tech, 40:135-141, 1986). Mannitol has also been found to provide little biological protection during freezing since it crystallizes and is thereby removed from the preparation (Gerald D. J. Adams, J. Richard Ramsay, Optimizing the lyophilization cycle and the consequences of collapse

> on the pharmaceutical acceptability of erwiniaL-Asparaginase, http://www3.interscience.wiley.com/cqi-bin/abstract/72505070/ABSTRACT). The suggestion of mannitol as a possible cryogenic protector does not show that mannitol is in fact successful and it was unpredictable from Ron whether or not the use of mannitol would be helpful in context with a lyophilized MP52 product. Though MP52 and BMP-2 belong to the same protein family, as previously pointed out, they do not exhibit identical physical behavior. Properties such as solubility cannot be transferred from one protein to another since individual amino acids on the protein surface have different hydrophobicity and can also show different solution behavior and different tendencies to aggregation. In general, it is not possible to transfer data from one protein to another even if they are in the same family. Thus, even if Ron had shown that mannitol acts as a cryogenic protector of BMP-2, which he did not, one would not assume that mannitol could also be used with MP-52. Since not all cryoprotectants can be used with all proteins, applicants contend that one skilled in the art would not reasonably expect mannitol to be useful with MP52 without testing. In view of the above discussion, applicants request that this rejection be withdrawn.

Applicants' arguments have been fully considered but they are not persuasive. The Williams reference is not of record. The examiner cannot consider evidence that is not of record. Furthermore, applicants have not explained the relevance of differences in solubility in basic amino acids, e.g. lysine, between BMP-2 and MP52 to lyophilization in mannitol.

The full Adams reference (Pharm Sci. 1996 Dec;85(12):1301-5) has been considered. Adams teaches:

> Solutes, added to formulations to "protect" sensitive bioproducts, may crystallize or remain as a glass (amorphous mass) when the solution is prefrozen. The precise response of a solute depends on its chemical nature, initial concentration, interaction with other solutes present, and freezing regime adopted.
>
> Eutectic freezing, where solvent (usually water) and solute crystallize completely to form an ice/solute crystal mixture, will dry with the sublimation of ice to leave a solid solute cake. Sensitive biomolecules may not be adequately protected when lyophilized in formulations consisting entirely of crystallizing solutes.

Page 1301, right column, full paragraphs 1 and 2.

> This report depicts the salient features of experimental techniques used at CAMR to define freeze-drying parameters. The essence of a formulation exercise should be to optimize cycle conditions to shorten drying times, while retaining essential properties of

> the pharmaceutical product including cake structure, biomolecule activity, and stability. Cycle development cannot be completed solely by defining collapse or melt conditions but must be accompanied by an experimental program which assesses biological properties. Using defined models, such as the enzyme L-asparaginase, formulations can be selected to enable successful optimization of processes. In this respect, formulations which readily crystallize and are satisfactory when processing robust chemicals may be inappropriate for freeze-drying labile biomolecules.

Page 1305, paragraph bridging left and right columns. However, there is no evidence of record that MP52 is a sensitive or labile biomolecule that would behave as Erwinia L-Asparaginase does during lyophilization. Therefore, Adams does not outweigh Ron's suggestion to lyophilize isolated or recombinant osteogenic proteins of the TGF-β family in the presence of mannitol. The Adams reference is not evidence that formulations containing mannitol would not be selected to enable successful optimization of MP52 lyophilization processes. Furthermore, obviousness does not require absolute predictability, only a reasonable expectation of success, i.e., a reasonable expectation of obtaining similar properties. Ron contains the suggestion to modify Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) to produce the claimed invention, and Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and Avis contain the evidence suggesting the modification would be successful. The references, taken as a whole, would have suggested applicants' invention to one of ordinary skill in the art at the time the invention was made.

Applicants argue that:

> As discussed in applicant's prior response, Chang does not cure the deficiencies in Neidhardt, Ron and Avis as Chang also shows that no general predictions can be made about the lyophilization conditions for specific proteins. Chang, on page 1325, first column, discloses that "despite the numerous freeze-thawing studies on proteins, the choice of these solutes and development of stable formulations is still largely empirical because of the lack of a full understanding of the relative importance of the various stresses arising during freezing and of mechanisms by which additives protect proteins against these stresses". In other words, for every protein, optimum conditions must be

determined individually and cannot be predicted from the results obtained with other proteins. In view of the above discussion, applicants request that this rejection be withdrawn.

Applicants' arguments have been fully considered but they are not persuasive. The examiner believes that the comments in Chang referred to by applicants are Chang's introductory comments directed to the problems which Chang addresses in the experiments that follow. Chang's results indicate that the freezing-induced denaturation is related to the exposure of proteins to an ice-water interface, so it seems rational to use surfactants as cryoprotectants (page 1327, right column, full paragraph 2). Chang teaches that the addition of small amounts of surface-active agents protected proteins from both freeze- and surface-induced denaturation (Abstract). The capacity of 0.01% Tween 80 to protect proteins during freeze-thawing appears to be quite general because all of the model proteins were essentially completely protected (paragraph bridging pages 1327-1328). To determine how general this protective effect was, the influence of several surfactants, with different chemical structures, on freeze denaturation of LDH was tested. All the tested surfactants protected LDH from precipitation during a quench-freezing process, even though the control frozen without a surfactant showed a significant increase in turbidity (Table 2). See page 1328, left column, full paragraph 1. The surfactants tested included Tween, Triton, and Brij. See page 1328, Table 2. This general stabilization of proteins during freeze-thawing by relatively low concentrations of surfactants strongly supports the contention that damage to proteins during freezing is due, at least to a large degree, to surface denaturation (page 1328, left column, full paragraph 2). A surfactant may only be sufficient to protect proteins during the freezing step. Another stabilizer, which is known to confer protection during drying (e.g., sucrose) will probably be needed to completely inhibit protein unfolding

during freeze-drying. See page 1329, right column, last paragraph. It is fair to say that Chang identifies the addition of surfactants and their combination with another stabilizer which is known to confer protection during drying, as a "result effective variable" for inhibiting protein denaturation during freeze-drying. Therefore, Chang contains specific guidance regarding the addition of surfactants during the freeze-drying process, a suggestion to modify Makishima (WO 96/33215) in view of Makishima (U. S. Patent No. 7,235,527) and further in view of Ron and Avis to produce the claimed invention, and evidence suggesting the modification would be successful.

Applicants point out that the argumentation in applicant's prior response regarding mixing ratios included a clerical error. Applicants remarks stated that "22 mg of BMP-2 and 8 mg of mannitol were used, i.e. a mixing ratio of 1:364". This should have stated "22 μg of BMP-2". In any case, a skilled artisan would have assumed that BMPs are used with considerably higher dosages of mannitol than is the case according to the present invention with MP52 wherein the optimum mixing ratio from 1:5-50 is sufficient. Applicants' arguments have been fully considered but they are not persuasive. The examiner believes he correctly construed applicants' previous arguments as being directed to "22 μg of BMP-2". Avis teaches that mannitol has been found to be most useful to increase the solids content of the original solution to between approximately 5 and 25% so that the freeze-dried product plug occupies essentially the same volume as that of the original solution (page 1566, column 2, full paragraphs 1-3). A 5 to 25% mannitol solution contains 50 to 250 mg mannitol per ml. A solution comprising 2 to 4 mg/ml MP52 and 50 to 250 mg/ml mannitol is a solution comprising MP52 and mannitol in the

range of 1 : 5-50 (ratio by weight). A lyophilized form of said solution is a composition comprising MP52 and mannitol in the range of 1 : 5-50 (ratio by weight).

Applicants argue that:

> As discussed above, the combination of Neidhardt, Ron, Avis and Chang, does not suggest that mannitol should be used when lyophilizing MP52. Hansen is cited only for the disclosure of surfactants for stabilization of freeze-dried proteins and does not cure the deficiencies in Neidhardt, Ron, Avis and Chang regarding the use of mannitol with MP52 in a lyophilized composition. In view of the above discussion, applicants request that this rejection be withdrawn.

Applicants' arguments have been fully considered but they are not persuasive. Hansen was not cited by the examiner in order to cure any alleged deficiencies in Neidhardt, Ron, Avis and Chang regarding the lyophilization of MP52 with mannitol. The examiner believes that he has already adequately addressed these alleged deficiencies. One of ordinary skill in the art would be motivated to modify Makishima (WO 96/33215), Ron, Avis and Chang with Hansen because further stabilization of freeze-dried proteins can be obtained by the addition of surfactants, such as poloxamers, as taught by Hansen.

Conclusion

No claims are allowable.

/DAVID ROMEO/
PRIMARY EXAMINER
ART UNIT 1647

DSR
AUGUST 6, 2007